

Make Your Own Freeze Media

By Rhonda Noël, MT(ASCP), NPHL

To preserve bacterial isolates for future use, prepare a freeze matrix and aliquot to sterile cryovials (with O-ring cap is best). Use LB (Luria-Bretani) broth, BHI (Brain Heart Infusion) broth, or TSB (Tryptic Soy Broth) and add sterile glycerol to create a final concentration of 30% sterile glycerol (v/v) as the matrix. The glycerol is heavy so mix well when working. Aseptically add about 1mL of the mix to the cryovials and store at 4°C. After inoculation with a loop of fresh culture, freeze the same vial at -20°C. The matrix won't freeze completely hard but remains slushy due to the glycerol.

To subculture from frozen stock, stab a hot loop into the vial while keeping the sample as cold as possible. Streak the inoculum to the appropriate plate medium. Immediately refreeze the vial. Freeze thaw cycles decrease the viability of the bacteria. Complete thawing of the sample may require a new preparation from a fresh subculture.

Additional information about cryopreservation of microorganisms can be found at the ATCC website (www.atcc.com).

Please call Rhonda Noël if you have questions, 402-559-7766.